

In the Specification:

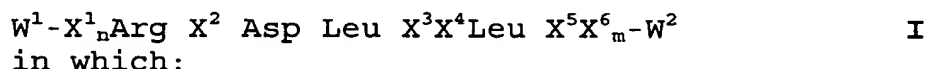
Please amend the specification as shown:

Please delete the paragraph on page 1, lines 3-11 and replace it with the following amended paragraph:

The invention describes novel peptides which, as ligands of the integrin $\alpha_v\beta_6$, are biologically active. These peptides all have a common structural motif, namely - Asp Leu Xaa Xaa Leu - , or in a preferred form - Arg Xaa Asp Leu Xaa Xaa Leu Arg - (SEQ ID NO: 1), where Xaa is any desired amino acid residue. The peptides according to the invention can be employed as effective inhibitors of the $\alpha_v\beta_6$ integrin receptor and thus for the treatment of various diseases and pathological findings.

Please delete the paragraph bridging pages 3-4 and replace it with the following amended paragraph:

The invention thus relates to peptide compounds of the formula I (SEQ ID NO: 2)



X^1 , X^2 , X^3 , X^4 , X^5 , X^6 each independently of one another are an amino acid residue, the amino acids independently of one another being selected from a group consisting of Ala, Asn, Asp, Arg, Cys, Gln, Glu, Gly, Phe, His, Ile, Leu, Lys, Met, Nle, homo-Phe, Phg, Pro, Ser, Thr, Trp, Tyr or Val, and the amino acids mentioned possibly also being derivatized,

W^2 is selected from a group OH, OR, NHR, NR_2 , NH_2 ,

W^1 is H or an acyl radical

R is alkyl having 1-6 C atoms and

n, m each independently of one another are a number from 0-15. In the cases in which m or n assumes a value of greater than 1, the radicals X^1 and X^6 can each independently of one another be identical or different.

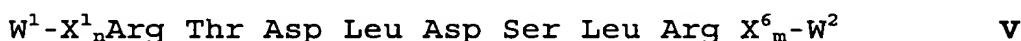
Please delete the paragraph bridging pages 4-5 and replace it with the following amended paragraph:

The preferred compounds (for meanings or abbreviations see above and below) thus include those of the general formula II

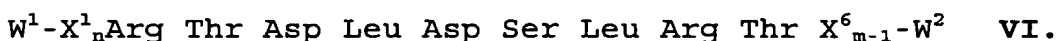
$W^1-X^1_n\text{Arg Thr Asp Leu } X^3X^4\text{Leu Arg } X^6_m-W^2$	<u>IIa, (SEQ ID NO: 3)</u>
$W^1-X^1_n\text{Arg Ser Asp Leu } X^3X^4\text{Leu Arg } X^6_m-W^2$	<u>IIb, (SEQ ID NO: 4)</u>
$W^1-X^1_n\text{Arg Asp Asp Leu } X^3X^4\text{Leu Arg } X^6_m-W^2$	<u>IIc, (SEQ ID NO: 5)</u>
$W^1-X^1_n\text{Arg Ser Asp Leu } X^3X^4\text{Leu Arg } X^6_m-W^2$	<u>IId, (SEQ ID NO: 6)</u>
$W^1-X^1_n\text{Arg Gly Asp Leu } X^3X^4\text{Leu Arg } X^6_m-W^2$	<u>IIe, (SEQ ID NO: 7)</u>
and those of the general formula III	
$W^1-X^1_n\text{Arg } X^2\text{Asp Leu Asp } X^4\text{Leu Arg } X^6_m-W^2$	<u>IIIa, (SEQ ID NO: 8)</u>
$W^1-X^1_n\text{Arg } X^2\text{Asp Leu Glu } X^4\text{Leu Arg } X^6_m-W^2$	<u>IIIb, (SEQ ID NO: 9)</u>
$W^1-X^1_n\text{Arg } X^2\text{Asp Leu Arg } X^4\text{Leu Arg } X^6_m-W^2$	<u>IIIc, (SEQ ID NO: 10)</u>
$W^1-X^1_n\text{Arg } X^2\text{Asp Leu Lys } X^4\text{Leu Arg } X^6_m-W^2$	<u>IIId, (SEQ ID NO: 11)</u>
$W^1-X^1_n\text{Arg } X^2\text{Asp Leu His } X^4\text{Leu Arg } X^6_m-W^2$	<u>IIIe, (SEQ ID NO: 12)</u>
$W^1-X^1_n\text{Arg } X^2\text{Asp Leu Tyr } X^4\text{Leu Arg } X^6_m-W^2$	<u>IIIf, (SEQ ID NO: 13)</u>
and those of the general formula IV	

$W^1-X^1_n\text{Arg } X^2\text{Asp Leu } X^3\text{Ser Leu Arg } X^6_m-W^2$	<u>IVa, (SEQ ID NO: 14)</u>
$W^1-X^1_n\text{Arg } X^2\text{Asp Leu } X^3\text{Tyr Leu Arg } X^6_m-W^2$	<u>IVb, (SEQ ID NO: 15)</u>
$W^1-X^1_n\text{Arg } X^2\text{Asp Leu } X^3\text{Thr Leu Arg } X^6_m-W^2$	<u>IVc, (SEQ ID NO: 16)</u>
$W^1-X^1_n\text{Arg } X^2\text{Asp Leu } X^3\text{Gly Leu Arg } X^6_m-W^2$	<u>IVd, (SEQ ID NO: 17)</u>
$W^1-X^1_n\text{Arg } X^2\text{Asp Leu } X^3\text{Val Leu Arg } X^6_m-W^2$	<u>IVe, (SEQ ID NO: 18)</u>

Particularly preferred peptide compounds according to the invention are those of the formula V (SEQ ID NO: 19)



and in this context in particular those of the formula VI (SEQ ID NO: 20)



Finally, the following individual compounds are particularly preferred, those also being included which are modified at the N and C termini: (SEQ ID NOS 21-28, respectively, in order of appearance)

- (a) H-Arg-Thr-Asp-Leu-Asp-Ser-Leu-Arg-Thr-Tyr-Thr-Leu-OH
- (b) H-Arg-Thr-Asp-Leu-Asp-Ser-Leu-Arg-OH
- (c) Ac-Arg-Thr-Asp-Leu-Asp-Ser-Leu-Arg-Thr-OH
- (d) Ac-Arg-Thr-Asp-Leu-Asp-Ser-Leu-Arg-Thr-NH₂
- (e) H-Arg-Thr-Asp-Leu-Asp-Ser-Leu-Arg-Thr-OH
- (f) H-Arg-Thr-Asp-Leu-Asp-Ser-Leu-Arg-Thr-NH₂
- (g) H-Arg-Thr-Asp-Leu-Tyr-Tyr-Leu-Arg-Thr-Tyr-OH
- (h) Ac-Arg-Thr-Asp-Leu-Asp-Ser-Leu-Arg-NH₂

Please delete the paragraph bridging pages 16-17 and replace it with the following amended paragraph:

In principle, the preparation and purification was carried out by means of Fmoc strategy with protection of acid-labile side chains on acid-labile resins using a commercially obtainable continuous flow peptide synthesizer according to the details of Haubner et al. (J. Am. Chem. Soc. 118, 1996, 17703). In the following, the synthesis and purification is described by way of example for the peptide amide Ac-RTDLDSLRLR-NH₂ (SEQ ID NO: 28). For the synthesis of peptide acids, an o-chlorotrityl chloride resin (Novabiochem) was coated with the appropriate C-terminal Fmoc amino acid according to the manufacturer's instructions and used in the synthesis apparatus according to the manufacturer's instructions (Milligen). The principal steps are washing - Fmoc protective group removal - washing - coupling with the next Fmoc amino acid - capping (acetylation) - washing. If an N-terminal acylation is desired after the last amino acid coupling, this is carried out after removal of the last Fmoc protective group using the appropriate activated acyl radical, e.g. the acetic anhydride. 2 g of 9-Fmoc-aminoxanthyloxy resin (Novabiochem, 0.37 mmol/g) were subjected to a coupling step, for 60 min in each case, in succession with 0.45 g each of hydroxybenzotriazole hydrate (HOBt), 0.5 ml of ethyldiisopropylamine, 4 equivalents each of diisopropylcarbodiimide (DIC) and Fmoc-amino acid in dimethylformamide (DMF), in a commercial synthesis apparatus and a typical procedure (apparatus and Milligen 9050 PepSynthesizerTM Handbook, 1987). Washing steps were carried out in DMF for 10 min, removal steps in piperidine/DMF (1:4 vol) for 5 min, N-terminal acetylations (capping) were carried out for 15 min using acetic anhydride/pyridine/DMF (2:3:15 vol). The amino acids Fmoc-Arg (Pmc), then Fmoc-Leu, then Fmoc-Ser(But), then Fmoc-Asp(OBut), then Fmoc-Leu, then Fmoc-Asp(OBut), then Fmoc-Thr(But), and finally Fmoc-Arg(Pmc) were used. After washing with DMF and isopropanol and subsequent drying in vacuo, 3.48 g of the N-terminally acetylated peptidyl resin, Ac-Arg(Pmc)-Thr(But)-Asp(OBut)-Leu-Asp(OBut)-Ser(But)-Leu-Arg(Pmc)-aminoxanthyloxy resin (SEQ ID NO: 29), were obtained.

Please delete the paragraph on page 17, lines 10-31 and replace it with the following amended paragraph:

By treatment of this peptidyl resin with trifluoroacetic acid/anisole/dichloromethane (74 ml/3.7 ml/ 74 ml) for 4 h at room temperature, filtration, concentration in vacuo and trituration with diethyl ether, it was possible to obtain a

precipitate of 0.6 g of peptide, Ac-Arg-Thr-Asp-Leu-Asp-Ser-Leu-Arg-NH₂ (SEQ ID NO: 28). Purification of the product was carried out by RP-HPLC on Lichrosorb RP18 (250-25, 7 μ m, Merck KGaA) in 0.3% TFA using a gradient of 4% on 24% 2-propanol in 2 h at 8 ml/min and assessment by means of a UV flow-through photometer at 215 nm.

The product-containing fractions were freeze-dried. According to FAB-MS (Fast Atom Bombardment Mass Spectroscopy), the product obtained corresponded to the expectations: C₄₁H₇₃N₁₅O₁₅M 1015.5 g/mol; (M+H)⁺ is 1016. In the analytical HPLC on SuperSpher RP18e (250-4, Merck KGaA) in a gradient of 0-99% A (0.08 M phosphate pH 3.5, 15% acetonitrile) to B (0.03 M phosphate pH 3.5, 70% acetonitrile) in 50 min, at 1 ml/min, and detection at 215 nm, the purified product Ac-Arg-Thr-Asp-Leu-Asp-Ser-Leu-Arg-NH₂ (SEQ ID NO: 28) has a retention time of 7.22 min.

Please delete the paragraph on page 18, lines 6-8 and replace it with the following amended paragraph:

Example 2

The following peptides shown in Table 1 were prepared and purified analogously to Example 1 (SEQ ID NOS 30-48, respectively, in order of appearance).

Please delete the paragraph on page 18 lines 12-14 and replace it with the following amended paragraph:

The comparison compounds used were known RGD peptides such as GRGDSPK (SEQ ID NO: 49), cyclo-(RGDfV) (SEQ ID NO: 50), and the linear peptide DLYYLM DL (SEQ ID NO: 51).

Please delete the paragraph on page 20, lines 9-18 and replace it with the following amended paragraph:

The prepared peptides according to the invention were bonded to the immobilized $\alpha_v\beta_6$ receptor in solution together with competitively acting fibronectin and the Q value was determined as a measure of the selectivity of the binding of the peptide to be tested to $\alpha_v\beta_6$. The Q value is in this case calculated from the quotient of the IC₅₀ values of test peptide and a standard. The standard used was the linear hepta-RGD peptide GRGDSPK (SEQ ID NO: 49) (ref./Patent cf. Pytela et al. Science 231, 1559, (1986)).

Please delete the paragraph bridging pages 20-21 and replace it with the following amended paragraph:

The immobilization of soluble $\alpha_v\beta_6$ receptor on microtitre plates was carried out by dilution of the protein solution in TBS++ and subsequent incubation overnight at 4°C (100 μ l/well). Non-specific binding sites were blocked by incubation (2 h, 37°C) with 3% (w/v) BSA in TBS++ (200 μ l/well). Excess BSA was removed by washing three times with TBSA++. Peptides were serially diluted (1:10) in TBSA++ and incubated with the immobilized integrin (50 μ l of peptide + 50 μ l of ligand per well; 2 h; 37°C) together with biotinylated fibronectin (2 μ g/ml). Unbound fibronectin and peptides were removed by washing three times with TBSA++. The detection of the bound fibronectin was carried out by incubation (1 h; 37°C) with an alkaline phosphatase-coupled anti-biotin antibody (Biorad) (1:20,000 in TBSA++; 100 μ l/well). After washing three times with TBSA++, the colorimetric detection was carried out by incubation (10-15 min; 25°C, in the dark) with substrate solution (5 mg of nitrophenyl phosphate, 1 ml of ethanolamine, 4 ml of H₂O; 100 μ l/well). The enzyme reaction was stopped by addition of 0.4 M NaOH (100 μ l/well). The colour intensity was determined at 405 nm in an ELISA measuring apparatus and made equal to the zero value. Wells which were not coated with receptor were used as a zero value. The standard employed was GRGDSPK (SEQ ID NO: 49). The IC₅₀ values for the tested peptides were read off from a graph and the Q value of the peptide according to the invention was determined from this together with the IC₅₀ value of the standard peptide. The results of the test described are summarized in the following table:

Please delete Table 2 on page 21 replace it with the following amended table:

Table 2

Structure	Q value = IC ₅₀ test peptide/ IC ₅₀ standard peptide
GRGDSPK (SEQ ID NO: 49)	1.0 (IC ₅₀ = 400 nM)
cyclo-(RGDfV) (SEQ ID NO: 50)	0.6
DLYYLMDL (SEQ ID NO: 51)	Inactive (IC ₅₀ >50 μ M)
RTDLDSLRTYTL (SEQ ID NO: 30)	0.27
DSLRTYTL (SEQ ID NO: 31)	Inactive (IC ₅₀ >50 μ M)
RRDLDSL (SEQ ID NO: 32)	2.5
DLDSLRTY (SEQ ID NO: 33)	Inactive (IC ₅₀ >50 μ M)
RTDLDSL (SEQ ID NO: 34)	0.17
RTDLDSLRTY (SEQ ID NO: 35)	0.10

Ac-RTDLDSLRT (<u>SEQ ID NO: 36</u>)	0.029
RTDLDSLRT (<u>SEQ ID NO: 37</u>)	0.11
RTDLDLRT-NH ₂ (<u>SEQ ID NO: 39</u>)	1.1
Ac-RTDLDLRT-NH ₂ (<u>SEQ ID NO: 40</u>)	0.5
RTDLYYLMDL (<u>SEQ ID NO: 41</u>)	0.33
RTDLDSLRT-NH ₂ (<u>SEQ ID NO: 42</u>)	0.056
RTDLDPRLTY (<u>SEQ ID NO: 43</u>)	0.50
RTDLYYLRTY (<u>SEQ ID NO: 44</u>)	0.042
Ac-RTDLDSLRT-NH ₂ (<u>SEQ ID NO: 45</u>)	0.013
TDLDSLRT (<u>SEQ ID NO: 47</u>)	66
PVDLYYLMDL (<u>SEQ ID NO: 48</u>)	Inactive (IC ₅₀ >50 μ M)